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# The Effect of Supplemental Energy, Nitrogen, and Protein on Feed Intake, Digestibility, and Nitrogen Flux Across the Gut and Liver in Sheep Fed Low-Quality Forage[1]

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**ABSTRACT:** Our objective was to determine the impact of supplemental energy, N, and protein on feed intake and N metabolism in sheep fed low-quality forage. Six Texel × Dorset wethers (16 mo, 63 ± 3.1 kg) fitted with mesenteric, portal, and hepatic venous catheters were used in a Latin square design with five sampling periods. Lambs were fed chopped bromegrass hay (4.3% CP) to appetite, and a mineral mixture was given. Treatments were 1) control (no supplement), 2) energy (cornstarch, molasses, and soybean oil), 3) energy plus urea, 4) energy plus soybean meal (SBM), and 5) energy plus ruminally undegraded protein (RUP; 50:50 mixture of blood and feather meals). Supplements were fed once daily (.3% BW). Forage DMI did not differ ($P$ = .13), but intake of total DM, N, and energy differed ($P$ < .01) among treatments. Apparent digestibilities of DM, OM, and energy were less ($P$ < .01) for control than for other treatments. Apparent N digestibility was least for control and energy and greatest for urea treatments ($P$ < .05). As a result, digested DM, OM, and energy ranked from least to greatest were control, energy, urea, SBM, and RUP, respectively. Apparently digested N was 2.44, 2.24, 11.39, 9.80, and 11.25 g/d for control, energy, urea, SBM, and RUP ($P$ < .01; SE = .10). Hour of sampling × treatment was a significant source of variation for blood concentrations of ammonia N and urea N, net ammonia N release from portal-drained viscera (PDV) and liver, and urea N release from splanchnic tissues. These results were primarily because patterns through time for the urea treatment differed from the other treatments. Net PDV release of $\alpha$-amino N did not differ ($P$ > .05) between control and energy treatments. Values for those treatments were about one-half of values for urea, SBM, and RUP treatments, which did not differ ($P$ > .05). Hepatic net uptake (negative release) of $\alpha$-amino N for control was 53% of values for the other treatments, which did not differ ($P$ > .05). Net release of $\alpha$-amino N from splanchnic tissues did not differ among treatments ($P$ = .34) and did not differ from zero. The data indicate that arterial $\alpha$-amino N concentration, hepatic $\alpha$-amino N uptake, PDV release and hepatic uptake of ammonia N, and hepatic release of urea N were greater in energy than in control treatments. We also found that hepatic uptake of $\alpha$-amino N was 187% of PDV release in energy-supplemented lambs. These results suggest that energy supplementation of a protein-limiting diet stimulated mobilization of body protein.

Key Words: Sheep, Forage, Nitrogen Metabolism

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## Introduction

When ruminants consume low-quality forage, animal performance and(or) forage utilization are improved with supplementation. This may be 1) due to greater energy intake with the consumption of a high-concentrate supplement, 2) because the protein portion provides the ruminal microflora with additional ammonia N and amino acids, and 3) because a portion of the protein escapes ruminal degradation and supplies additional amino acids to the small intestine.

Ruminal degradation of protein in feedstuffs varies from 20 to 80% (NRC, 1988). When a ruminally undegraded protein (**RUP**) was fed, more amino acids flowed to the duodenum and disappeared in the small intestine than when soybean meal was fed (Titgemeyer et al., 1989). Also, in sheep limit-fed forage diets, feeding RUP increased net portal-drained viscera (**PDV**) release of $\alpha$-amino N (Cleale et al., 1987). Conversely, in cattle limit-fed a 60% concentrate diet, varying the level of

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Table 1. Ingredient composition of mineral mix, DM basis[a]

| Ingredient | Percentage of mineral mix |
|---|---|
| Salt | 21.23 |
| Dicalcium phosphate | 43.69 |
| $MgSO_4 \cdot 7H_2O$ | 26.10 |
| Vitamin ADE premix[b] | 2.17 |
| Vitamin E premix[c] | 2.17 |
| Trace mineral premix[d] | 2.17 |
| Soybean oil | 2.17 |

[a]Fed to wethers at 22 g/d.
[b]Each gram of premix contained 8,800 IU of vitamin A, 880 IU of vitamin D, and .88 IU of vitamin E.
[c]Each gram of premix contained 44 IU of vitamin E.
[d]Contained 14% Ca, 12% Zn, 8% Mn, 10% Fe, .2% I, and .1% Co.

dietary RUP had no effect on net PDV $\alpha$-amino N absorption (Huntington, 1987). Extrapolating the absorption data to a production situation is difficult, because ruminant animal production usually involves feeding animals for maximum intake. Our objective for this study was to determine how supplemental energy, energy plus N, and source of N affect PDV and hepatic N metabolism in sheep consuming low-quality forage ad libitum.

## Experimental Procedures

*Sheep.* This experiment was approved by the U.S. Meat Animal Research Center Animal Care and Use Committee. The Texel × Dorset wethers were 16 mo old and weighed 63 ± 3.1 kg (range of 52 to 73 kg) at the start of the experiment. They were surgically fitted with blood sampling catheters placed in a mesenteric artery, mesenteric vein, portal vein, and a hepatic vein as previously described (Ferrell et al., 1991). The catheters were placed in the sheep when they were 8 mo old, and they were used in a previous experiment (Freetly et al., 1995). Mesenteric arterial, mesenteric venous, and hepatic venous catheters were functional in all six sheep, and portal venous catheters were functional in five of six sheep. The wethers were housed in a metabolism room with a 12-h light:dark cycle, and temperature was maintained at 20°C. They were placed individually in elevated pens (1.2 $m^2$) equipped with wire mesh slatted floors (.63- × 3.5-cm slots). Nipple waterers were on the inside and a feed bunk was on the outside of each pen.

*Experimental Design.* Six wethers were used in a Latin square design consisting of the six sheep and five sampling periods (Cochran and Cox, 1957). They were offered chopped brome hay (ground with a tub grinder equipped with a 10-cm screen) to appetite, and a loose mineral was fed at 22 g/d (Table 1). Dietary treatments consisted of no supplement (control) or once-daily supplementation with energy, energy plus urea (urea), energy plus soybean meal (**SBM**), or energy plus ruminally undegraded protein (RUP; Table 2).

Supplements were formulated and fed as follows. Energy supplement was designed to be low-protein, high-energy, and palatable. It was fed at a rate such that the mineral mix plus energy supplement was consumed at .30% of BW/d. Urea supplement was formulated to contain 30% CP using urea, which was substituted for cornstarch and dried molasses on a proportional basis. Urea supplement was offered at 10% greater intake than the energy supplement so that the amount of molasses and cornstarch consumed was identical between energy- and urea-supplemented sheep. For SBM and RUP, the amount of dried molasses was held constant, the protein sources (SBM and RUP) were substituted for cornstarch, and all three supplements were fed at the same rate (percentage of BW). For RUP, 50% of the added protein was supplied from blood meal and 50% from feather meal. The CP requirement for maintenance of a 60-kg ewe is 104 g/d (NRC, 1985). Crude protein intake ranged from 47 to 50 g/d (N × 6.25; Table 3) in control wethers and those supplemented with energy. In wethers supplemented with urea, SBM, or RUP, CP intake ranged from 103 to 117 g/d. Therefore, in controls and energy-supplemented sheep, CP intake was about half their requirement for maintenance, whereas CP intake of urea-, SBM-, or RUP-supplemented sheep was near the requirement for maintenance.

*Feeding and Sampling.* At 0700, lights came on and feed refused (hay) was removed, weighed, and discarded. At 0900 sheep were fed mineral and supplement, and at 0930 they were fed chopped brome hay. The amount of hay offered varied between 115 and 125% of the previous 3-d forage intake. With this feeding protocol, the mineral and supplement were completely consumed before hay was offered.

Each of five periods of the Latin square lasted 21 d, with d 1 to 13 for adaptation to dietary treatment. On d 14 to 19, sheep were fitted with fecal bags and harnesses. Feces were collected daily between 0730 and 0900, weighed, subsampled (10%), and stored at 2°C. At the end of each period, feces were composited by sheep and stored frozen (–20°C). On d 20 and 21, blood sampling occurred, sampling three sheep each day. On d 21 at 1600, ruminal fluid (25 mL) was collected using a stomach tube. It was acidified by combining 2 mL of .2 *N* HCl with 8 mL of ruminal fluid and stored frozen at –20°C. When ruminal fluid was stored frozen for later ammonia analysis, preserving it with .2 *N*, .5 *N*, or .6 *N* acid resulted in less ammonia loss compared with storing it in .05 *N* acid (Nocek et al., 1987).

During blood sampling, sheep were placed in portable crates (40 × 117 cm) at 0700. They had water available, and their feeding schedule was similar to that just described. At 0730, we began infusing a primed (15 mL) continuous infusion (.6 mL/min) of 4% (wt/vol) para-aminohippuric acid (**PAH**) into the mesenteric venous catheter. Ten milliliters of blood was drawn simultaneously from the mesenteric arterial, portal venous, and hepatic venous catheters into heparinized syringes and

Table 2. Ingredient composition of supplements and nutrient analyses, DM basis

| | Supplement[a] | | | |
|---|---|---|---|---|
| Ingredient | Energy | Urea | SBM | RUP |
| Dried molasses | 33.33 | 30 | 30 | 30 |
| Cornstarch | 64.67 | 57.97 | 10.45 | 36.66 |
| Urea | 0 | 10.03 | 0 | 0 |
| Soybean meal | 0 | 0 | 57.55 | 0 |
| Blood meal | 0 | 0 | 0 | 15.67 |
| Feather meal | 0 | 0 | 0 | 15.67 |
| Soybean oil | 2 | 2 | 2 | 2 |
| Nutrient analyses[b] | | | | |
| CP, % (N × 6.25) | 3.3 | 33.6 | 29.9 | 34.4 |
| Ash, % | 3.6 | 3.2 | 7.1 | 4.3 |
| NDF, % | 9.3 | 7.8 | 16.1 | 17.2 |

[a]SBM = soybean meal; RUP = ruminally undegraded protein.
[b]Brome hay contained 4.3% CP, 8.2% ash, and 73.9% NDF.

placed on ice. Blood samples were collected at 0800, 0820, 0840, 0900, and then hourly for 6 h more. On blood sampling days, supplement was consumed within 30 min, and forage consumption also appeared normal. For 2 d before and the day of blood sampling, daily forage intake averaged 993, 974, 1,078, 1,102, and 1,121 g/d for control and energy-, urea-, SBM-, and RUP-supplemented sheep, respectively.

*Laboratory Analyses.* Dry matter of feed and feces was determined by drying in a forced-air oven at 60°C for 48 h. Samples were ground (1-mm screen) and assayed for ash (AOAC, 1975), N (AOAC, 1976), and NDF (Goering and Van Soest, 1970). Within 2 h after blood sampling, 1 mL of whole blood was combined with 3 mL of deionized, distilled water and assayed for PAH (Harvey and Brothers, 1962), $\alpha$-amino N (Broderick and Kang, 1980), ammonia N (Ilmer et al., 1972), and urea N (Marsh et al., 1965) using a Technicon Autoanalyzer System (Technicon Autoanalyzer System, Tarrytown, NY). Because the ammonia N reaction is pH-sensitive (Beecher and Whitten, 1970), standards were made in 4% (vol/vol) HCl, when ruminal fluid was assayed for ammonia N concentration. Nutrient flux was calculated as outlined by Burrin et al. (1991).

*Statistical Analysis.* Feed intake and nutrient digestibility data were analyzed as an incomplete Latin square design using GLM procedures of SAS (1990). The model included sheep, period, and supplement. Supplement means were compared by use of F-protected *t* statistics.

Nitrogen-containing components in whole blood and flux across the gut and liver were analyzed using a split-plot analysis. The model included dietary treatment (**Trt**), sheep, and period, hour after feeding, and the hour after feeding × Trt interaction. Sums of squares due to Trt × sheep × period were used as the whole-plot error term to test for significance of supplementation effects. The residual sums of squares were used as the subplot error term to test for significance of hour after feeding and hour after feeding × supplement interaction. Dietary treatment means were compared by use of F-protected *t* statistic.

## Results and Discussion

*Feed Intake and Digestibility.* The mineral mix was fed at 20.8 g DM daily to all treatment groups (Table 3). Sheep fed urea, SBM, and RUP were fed 10% more supplement than sheep fed energy to maintain equal energy intakes from supplements across supplemental treatments. In addition, diets were designed such that supplemental N was constant across urea, SBM, and RUP treatments. The bromegrass hay, consumed ad libitum, contained (mean ± SD, n = 5) 90.7 ± 1.3% DM, 8.24 ± .27% ash, 4.375 ± 20 kcal/g gross energy, 4.32 ± .66% CP, and 73.88 ± .61% NDF. Forage intake did not differ significantly ($P = .13$) among treatments, even though forage intake by RUP-supplemented sheep was 109, 114, 109, and 107% of that of control and energy-, urea-, and SBM-supplemented sheep, respectively. Total DMI differed ($P < .001$) among treatments. Total DMI for energy-supplemented sheep was greater than for control ($P < .05$) but was less than for RUP ($P < .05$). Total DMI for urea and SBM treatments were intermediate between energy and RUP treatments. Total organic matter and energy intakes followed similar patterns. In contrast, N intake was lower for the control and energy-supplemented sheep than for N-supplemented sheep, as designed. Among N-supplemented sheep, those fed SBM consumed the least N and those fed RUP consumed the most.

Protein supplementation of ruminant livestock that consume low-quality forage is expected to increase BW or reduce BW loss. These effects are usually attributed to increased voluntary intake or diet digestibility. In this study, forage intake was not influenced substantially by supplementation. Although forage intake is often depressed when high-energy, low-protein supplements are used and is generally increased when low-energy, high-protein supplements are provided, ob-

Table 3. Effect of supplementation on feed intake and nutrient digestibility in wethers consuming poor-quality bromegrass hay ad libitum

| | Treatment means[a] | | | | | | | Probability[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | Control | Energy | Urea | SBM | RUP | $\overline{X}$ | SE | Sheep | Period | Treatment |
| No. of observations | 6 | 6 | 6 | 6 | 6 | | | | | |
| BW, kg | 63.0 | 64.0 | 63.6 | 63.6 | 64.3 | 63.7 | .31 | .001 | .22 | .47 |
| DMI, g/d | | | | | | | | | | |
| Mineral | 20.8 | 20.8 | 20.8 | 20.8 | 20.8 | | | | | |
| Supplement | 0 | 172 | 189 | 188 | 191 | | | | | |
| Forage | 1,092 | 1,039 | 1,090 | 1,107 | 1,188 | 1,103 | 22.4 | .001 | .03 | .13 |
| Total | 1,114$^{c}$ | 1,232$^{d}$ | 1,300$^{de}$ | 1,316$^{de}$ | 1,400$^{e}$ | 1,272 | 22.2 | .002 | .04 | .001 |
| OM intake, g/d | 1,010$^{c}$ | 1,126$^{d}$ | 1,190$^{de}$ | 1,197$^{de}$ | 1,280$^{e}$ | 1,160 | 20.34 | .002 | .05 | .001 |
| N intake, g/d | 7.53$^{c}$ | 8.04$^{c}$ | 17.67$^{e}$ | 16.65$^{d}$ | 18.69$^{f}$ | 13.72 | .20 | .02 | .21 | .001 |
| Energy intake,Mcal/d | 4.78$^{c}$ | 5.26$^{d}$ | 5.54$^{de}$ | 5.68$^{ef}$ | 6.10$^{f}$ | 5.47 | .10 | .002 | .04 | .001 |
| Digestibility, % | | | | | | | | | | |
| DM | 50.4$^{c}$ | 55.0$^{d}$ | 54.6$^{d}$ | 54.3$^{d}$ | 55.1$^{d}$ | 53.9 | .52 | .14 | .14 | .007 |
| OM | 52.3$^{c}$ | 57.2$^{d}$ | 56.9$^{d}$ | 56.5$^{d}$ | 57.4$^{d}$ | 56.0 | .52 | .10 | .13 | .003 |
| N | 32.1$^{c}$ | 27.4$^{c}$ | 64.5$^{e}$ | 58.8$^{d}$ | 59.8$^{de}$ | 48.5 | 1.02 | .79 | .03 | .001 |
| Energy | 47.4$^{c}$ | 52.3$^{d}$ | 52.0$^{d}$ | 52.0$^{d}$ | 53.3$^{d}$ | 51.4 | .58 | .15 | .08 | .005 |
| Digested intake | | | | | | | | | | |
| DM, g/d | 559$^{c}$ | 678$^{d}$ | 711$^{de}$ | 715$^{de}$ | 772$^{e}$ | 687 | 15.0 | .01 | .10 | .001 |
| OM, g/d | 526$^{c}$ | 644$^{d}$ | 677$^{de}$ | 677$^{de}$ | 735$^{e}$ | 652 | 14.1 | .01 | .07 | .001 |
| N, g/d | 2.44$^{c}$ | 2.24$^{c}$ | 11.39$^{e}$ | 9.80$^{d}$ | 11.25$^{e}$ | 7.42 | .17 | .10 | .19 | .001 |
| Energy, Mcal/d | 2.26$^{c}$ | 2.75$^{d}$ | 2.89$^{de}$ | 2.96$^{de}$ | 3.25$^{e}$ | 2.82 | .07 | .02 | .11 | .001 |

[a]Supplements were: Control = mineral only; Energy = energy plus mineral; Urea = energy plus urea and minerals; SBM = soybean meal plus energy and minerals; and RUP = ruminally undegraded protein (50:50 mixture of blood and feather meals) plus energy and minerals.

[b]Sheep (df = 5), period (df = 4), and treatment (df = 4) were tested against residual error (df = 16).

[c,d,e]Within a row, means lacking a common superscript letter differ ($P < .05$).

served responses have not been totally consistent (Peterson, 1987). The basis for the inconsistencies has not been explained. Mertens (1987) proposed that NDF can be used to represent dietary fill and that, based on his earlier review of several studies (Mertens, 1985), the capacity of cows to consume daily intake of NDF is limited to approximately 1.2% of BW. Forage NDF consumed by lambs in this study was 1.30% (± .03) of BW and did not differ ($P$ = .28) among treatments. In contrast, data reported by Köster et al. (1996) indicated that forage NDF consumption by unsupplemented cows fed a poor-quality grass hay was only .5% of BW but increased to a maximum of 1.1% of BW with increasing levels of intraruminal casein infusion. Results of DelCurto et al. (1990) were similar to those of Köster et al. (1996) in that NDF consumption of unsupplemented control steers fed poor-quality prairie grass hay was approximately .6% of BW. In those studies, DMI or NDF consumption was generally increased by various supplementation protocols, but the maximum NDF intake observed was 1.27% of BW when a high-protein, low-energy supplement was provided. Forage NDF intake of steers fed a poor-quality grass hay with supplementary protein provided by varying mixtures of casein and urea (Köster et al., 1997) was approximately 1.0% of BW. These observations suggest that an intake response to supplementation may be expected if forage intake without supplementation is low, but that if forage intake without supplementation is relatively high then an increase in intake in response to supplementation is not likely. Factors affecting forage intake include fill, digestion rate, and passage rate, among others. These variables are influenced substantially by rates of microbial growth and fermentation. Microbial growth may be limited, particularly with some low-quality forages, by low ruminal ammonia concentrations. Satter and Slyter (1974) suggested that 3.6 m$M$ $NH_3$ N supported maximal microbial growth, but that limiting concentrations were perhaps closer to 1.5 m$M$. Ruminal $NH_3$ N concentrations observed in the present study were 2.5, 2.5, 6.6, 6.1, and 4.0 m$M$ (± .5) for control, energy, urea, SBM, and RUP treatments, respectively. Although we recognized that the sampling procedure used in the present study was inadequate to characterize ruminal ammonia profiles, these values suggest that microbial growth was likely not limited in the present study by ammonia availability in the rumen. In contrast, Köster et al. (1996) reported a value of .24 m$M$ in unsupplemented cows. The reason for the large difference in ruminal ammonia concentration between studies is not evident.

Apparent digestibility (Table 3) of DM, OM, and energy was greater ($P$ < .01) in supplemented sheep than in controls, but it did not differ ($P$ > .10) among supplements. If one assumes that apparent digestibility of forage and mineral DM was the same across all treatments (50.4%), DM digestibilities of energy, urea, SBM, and RUP supplements (estimated by difference) were 83.6, 80.0, 78.0, and 85.2%, respectively. Alternatively, if one assumes that apparent digestibilities of supplements were the same (81.7%), calculated digestibilities of forage and mineral DM were 50.7, 50.1, 49.8, and 51.0% for sheep fed energy, urea, SBM and RUP, respectively. Results from these simple calculations suggest that supplementation did not result in substantial changes in apparent DM digestibility of the forage used in this study. Most of the difference in digestibility of total diet by supplemented vs control sheep was attributable to the high digestibilities of the supplements.

Apparent digestibility of N tended ($P$ = .07) to be less in energy-supplemented than in control sheep, and, in those treatments, it was approximately 50% of that ($P$ < .01) in N-supplemented sheep (Table 3). Among N supplements, apparent N digestibility was greater for urea than for SBM ($P$ < .05) or RUP ($P$ = .07). The high fiber and low CP concentrations of low-quality forages are expected to result in low apparent digestibility of N because metabolic fecal N constitutes a high proportion of the N in the feces. The NRC (1985) suggested that metabolic fecal protein can be estimated as 33.44 g/kg DMI, whereas NRC (1988) suggested a value of 30 (with a range of 21 to 40) g/kg DMI. For control and energy-, urea-, SBM-, and RUP-supplemented sheep, use of 30 g/kg DMI yields values of 5.35, 5.91, 6.24, 6.32, and 6.72 (± .10) g metabolic fecal N/d, respectively. Observed total fecal N was 5.09, 5.80, 6.28, 6.85, and 7.44 (± .12) g/d. These values suggest that 90 to 105% of the observed fecal N loss was attributable to metabolic fecal N. We suggest that apparent digestibilities of N for these types of diets should be interpreted with caution.

As a result of greater total DMI and greater digestibility, digested DM, OM, and energy (Table 3) were approximately 22% greater ($P$ < .05) for energy-supplemented lambs than for controls. Digested DM, OM, and energy were numerically greatest for lambs supplemented with RUP; values were approximately 40% greater ($P$ < .05) than for controls and 13 to 18% greater ($P$ < .05) than for energy-supplemented lambs. Values for urea- and SBM-supplemented lambs were intermediate between those of lambs supplemented with energy or RUP. Neither N intake nor apparent digestibility differed between control and energy-supplemented lambs; thus, digested N was not different ($P$ = .61). Apparently digested N for urea-, SBM-, and RUP-supplemented lambs was 467, 402, and 461% of control values ($P$ < .05); both intake and apparent digestibility were greater when supplemental N was provided. The value for SBM was less ($P$ < .05) than for urea or RUP, which did not differ ($P$ > .05). The DE required for maintenance of a 60-kg sheep has been estimated to be approximately 2.7 Mcal/d (NRC, 1985). Thus, energy digested by control, energy, urea, SBM, and RUP treatments was approximately 84, 102, 107, 110, and 120% of that required for maintenance, respectively. Similarly, the estimated digestible CP required to maintain a 60-kg sheep has been estimated to be 53 g/d (NRC, 1975). Thus, digested CP values were approximately 29, 26,

134, 116, and 133% of estimated maintenance requirements.

*Blood Flow and Metabolite Flux.* Neither supplemental treatment ($P > .05$) nor the hour × treatment interaction ($P > .80$) was a significant source of variation in portal venous, hepatic arterial, or hepatic venous blood flow (Table 4). However, hour was a significant source of variation for portal ($P < .003$) and hepatic ($P < .01$) venous blood flow (data not shown). As expected, portal venous blood flow declined to a minimum immediately before feeding (107 L/h), increased to a maximum at 5 h after feeding (143 L/h), and then began to decline at 6 h (128 L/h). Although not statistically different, hepatic arterial blood flow was maximum before feeding (32 L/h) then declined to a minimum at 5 h (22.5 L/h) after feeding. The pattern of hepatic venous blood flow was similar to that of the portal venous, but changes through time were somewhat muted. In sheep that were limit-fed forage diets once daily (Bensadoun and Reid, 1962), portal blood flow increased to a maximum at 3 to 7 h postfeeding. Values at maximum averaged 41% greater than the mean. Although based on very limited numbers, those data suggested that form and amount of diet influenced the magnitude of the postprandial increase in portal blood flow. In cattle that were meal-fed twice daily (Whitt et al., 1996), portal and hepatic plasma flows were generally minimal immediately before feeding and increased after feeding to a maximum at 4 to 7 h after feeding. The largest observed deviations from the mean was 9% for portal and 8% for hepatic plasma flow.

*α-Amino Nitrogen.* Hour of sampling was a significant source of variation for concentration of α-amino N in arterial, portal venous, and hepatic venous blood (Table 4). Similar patterns were observed for all vessels. Concentrations (data not shown) were greatest before feeding (4.87, 4.60, and 4.63 m*M* in portal, hepatic, and arterial blood, respectively) then declined to a minimum (4.50, 4.27, and 4.28 m*M* in portal, hepatic, and arterial blood, respectively) at 3 to 6 h after feeding. The observed concentration patterns through time were, in general, the inverse of those observed for portal and hepatic blood flows. Supplemental treatment was not a significant source of variation in arterial ($P = .12$) and hepatic ($P = .13$) concentration of α-amino N. Concentrations were numerically higher in the energy-supplemented group than in the other groups. A similar pattern was observed for portal venous concentration, but the difference was not significant ($P = .28$). The reasons for these observations are not readily evident, but these observations lead us to speculate that blood concentrations of α-amino N may reflect body protein mobilization or status as well as net release from the portal-drained viscera (PDV).

Net release of α-amino N from the PDV was greater ($P < .05$) in sheep supplemented with urea, SBM, or RUP than in control sheep or those supplemented with energy (Table 4). In contrast, net uptake of α-amino N by hepatic tissues in energy-supplemented sheep was similar to that in urea-, SBM-, and RUP-supplemented sheep. Net uptake of α-amino N by hepatic tissues of both energy- and N-supplemented sheep were greater ($P < .05$) than that of controls. It is particularly noteworthy that, even though net portal release of α-amino N was similar, net uptake of α-amino N by the liver was nearly twofold greater in energy-supplemented sheep than in controls. Net uptake of α-amino N by splanchnic tissues did not differ from zero and did not differ ($P = .32$) among treatments, even though net uptake in the energy treatment seemed to be greater than in the others.

Burrin et al. (1991) fed ram lambs a high-quality diet for ad libitum intake and observed net uptake of α-amino N across splanchnic tissues. In cattle, the liver extracted a large portion of the net PDV release, but net splanchnic flux was still positive (Eisemann and Nienaber, 1990; Reynolds et al., 1992). In the present study, total flow of α-amino N to the liver was 571, 782, 733, 730, and 661 (residual SD [**RSD**] = 155) mmol/h for control, energy, urea, SBM, and RUP treatments ($P = .26$), respectively. The net release of α-amino N from the PDV averaged 2.66, 2.35, 4.42, 4.27, and 4.34% (RSD = 2.73) of the total α-amino N flowing to the liver for control, energy, urea, SBM, and RUP treatments, respectively ($P = .17$). Liver extraction ratios were 3.06, 3.88, 4.49, 3.90, and 5.67% (RSD = 2.46) for those treatments, respectively ($P < .06$). These observations indicate that net release of α-amino N from the PDV was a relatively minor contributor to the total amino acid N flowing to the liver and from splanchnic tissues. In addition, we suggest that energy supplementation of protein-limited sheep likely resulted in mobilization of body protein.

*Ammonia Nitrogen.* Hour of sampling × treatment interactions for ammonia N concentrations in hepatic venous ($P = .06$) and portal venous ($P < .05$), but not arterial ($P = .21$), blood were observed (Table 4). The interactions primarily resulted from the differing response through time of the urea-supplemented sheep compared with the other treatments. Ammonia N concentrations in arterial and hepatic venous were slightly elevated in sheep fed the urea supplement at 1 to 4 h after feeding compared with the other treatments (Figure 1). Portal venous ammonia N concentrations in sheep fed the urea supplement increased substantially during the h 1 after the supplement was fed, declined during 6 h after feeding, but had not returned to prefeeding levels at 6 h after feeding. Although not as dramatic, portal venous ammonia N concentrations in SBM-supplemented sheep were, as expected, elevated relative to the control, energy, and RUP treatments from 2 to 6 h after feeding.

Hour of sampling × treatment interactions ($P \leq .05$) were also observed for net PDV release and net hepatic uptake of ammonia N (Table 4). Net PDV release of ammonia N in the urea-supplemented sheep increased from an average of 13.6 mmol/h prefeeding to 39.1 mmol/h at 1 h after feeding, then subsequently declined

Table 4. Effect of supplementation on blood flow, nutrient concentration differences, and net nutrient release in sheep fed a low-quality forage[a]

| | Treatment means[b] | | | | | | | Probability[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | Control | Energy | Urea | SBM | RUP | Mean | SE | Treatment (T) | Sheep | Period | Hour (H) | H × T |
| Blood flow, L/h | | | | | | | | | | | | |
| Portal vein | 104 | 128 | 126 | 141 | 111 | 122.4 | 19.0 | .14 | .02 | .10 | .003 | .88 |
| Hepatic artery | 23 | 24 | 32 | 20 | 26 | 24.9 | 7.7 | .27 | .02 | .46 | .49 | .92 |
| Hepatic vein | 128 | 149 | 159 | 159 | 144 | 147.9 | 14.8 | .06 | .01 | .02 | .01 | .88 |
| $\alpha$-amino N concentration, m*M* | | | | | | | | | | | | |
| Artery | 4.39 | 4.84 | 4.41 | 4.40 | 4.39 | 4.46 | .24 | .12 | .32 | .19 | .001 | .97 |
| Portal vein | 4.55 | 4.98 | 4.70 | 4.60 | 4.64 | 4.68 | .27 | .28 | .17 | .15 | .001 | .97 |
| Hepatic vein | 4.36 | 4.74 | 4.39 | 4.40 | 4.33 | 4.44 | .23 | .13 | .24 | .15 | .001 | .99 |
| $\alpha$-amino N net release, mmol/h | | | | | | | | | | | | |
| Portal | 14.22[d] | 18.58[d] | 31.31[e] | 30.91[e] | 27.24[e] | 24.68 | 7.13 | .02 | .06 | .22 | .21 | .86 |
| Hepatic | –17.29[d] | –30.04[e] | –33.46[e] | –28.89[e] | –34.18[e] | –28.45 | 7.39 | .04 | .02 | .42 | .10 | .80 |
| Splanchnic | –4.05 | –10.68 | –3.48 | –.12 | –4.79 | –4.55 | 6.18 | .32 | .03 | .34 | .17 | .86 |
| Ammonia N concentration, m*M* | | | | | | | | | | | | |
| Artery | .160 | .170 | .172 | .157 | .144 | .160 | .012 | .04 | .07 | .001 | .001 | .21 |
| Portal vein | .242[d] | .268[de] | .344[f] | .284[e] | .261[de] | .281 | .036 | .02 | .32 | .48 | .001 | .004 |
| Hepatic vein | .153 | .166 | .165 | .150 | .136 | .154 | .011 | .03 | .06 | .001 | .001 | .06 |
| Ammonia N net release, mmol/h | | | | | | | | | | | | |
| Portal | 8.21[d] | 13.69[e] | 21.00[f] | 19.71[f] | 12.49[e] | 15.26 | 5.00 | .03 | .17 | .45 | .009 | .05 |
| Hepatic | –9.20[d] | –14.37[e] | –22.39[f] | –20.75[f] | –13.51[e] | –16.27 | 4.54 | .01 | .13 | .42 | .01 | .05 |
| Splanchnic | –.92 | –.77 | –1.11 | –1.07 | –.98 | –.94 | .62 | .93 | .45 | .78 | .16 | .54 |
| Urea N concentration, m*M* | | | | | | | | | | | | |
| Artery | 1.81[d] | 1.88[d] | 4.34[f] | 3.85[e] | 3.68[e] | 3.10 | .54 | .001 | .10 | .75 | .16 | .001 |
| Portal vein | 1.58[d] | 1.77[d] | 4.28[f] | 3.83[e] | 3.59[e] | 3.03 | .66 | .001 | .08 | .87 | .01 | .001 |
| Hepatic vein | 1.84[d] | 1.82[d] | 4.45[f] | 3.94[e] | 3.79[e] | 3.18 | .55 | .001 | .07 | .82 | .32 | .001 |
| Urea N net release, mmol/h | | | | | | | | | | | | |
| Portal | –19.85 | –20.58 | –28.20 | –28.90 | –12.91 | –23.37 | 11.07 | .33 | .18 | .22 | .28 | .59 |
| Hepatic | 26.52[d] | 40.31[e] | 65.46[f] | 56.89[f] | 45.93[e] | 47.48 | 12.42 | .04 | .16 | .15 | .92 | .28 |
| Splanchnic | 7.08[d] | 13.98[d] | 35.26[e] | 26.79[e] | 35.91[e] | 22.97 | 15.46 | .04 | .28 | .70 | .98 | .03 |
| Oxygen concentration, m*M* | | | | | | | | | | | | |
| Artery | 4.45 | 4.44 | 4.27 | 4.08 | 4.27 | 4.31 | .27 | .38 | .57 | .07 | .05 | .64 |
| Portal | 3.30[e] | 3.46[e] | 3.29[e] | 2.84[d] | 3.28[e] | 3.23 | .25 | .05 | .11 | .02 | .30 | .76 |
| Hepatic | 2.71 | 2.83 | 2.48 | 2.68 | 2.36 | 2.53 | .25 | .07 | .11 | .03 | .46 | .35 |
| Oxygen net release, mmol/h | | | | | | | | | | | | |
| Portal | –121.4 | –142.8 | –143.4 | –158.7 | –123.7 | –139.8 | 20.0 | .12 | .01 | .06 | .24 | .60 |
| Hepatic | –103.8[d] | –129.8[e] | –159.4[f] | –132.6[e] | –143.7[ef] | –132.3 | 17.5 | .01 | .05 | .24 | .50 | .88 |
| Splanchnic | –221.0[d] | –257.2[e] | –301.1[f] | –286.0[f] | –279.5[ef] | –268.3 | 22.8 | .01 | .01 | .01 | .05 | .85 |

[a]Positive numbers for net release indicate net output and negative numbers indicate net uptake.

[b]Supplements were: Control = minerals only; Energy = energy plus minerals; Urea = energy plus urea and minerals; SBM = soybean meal, energy and minerals; and RUP = ruminally undegraded protein (blood and feather meals) plus energy and minerals.

[c]Mean square for treatment (df = 4), sheep (df = 5), and period (df = 4) were tested against mean squares for treatment × sheep × period (df = 16); SE was calculated as √ mean square/n for the three-way interaction. Hour (df = 9) and hour × treatment (df = 36) were tested against residual error (df = 215).

[d,e,f,g]Within a row, means lacking a common superscript letter differ ($P < .05$) as determined by protected *t*-test.

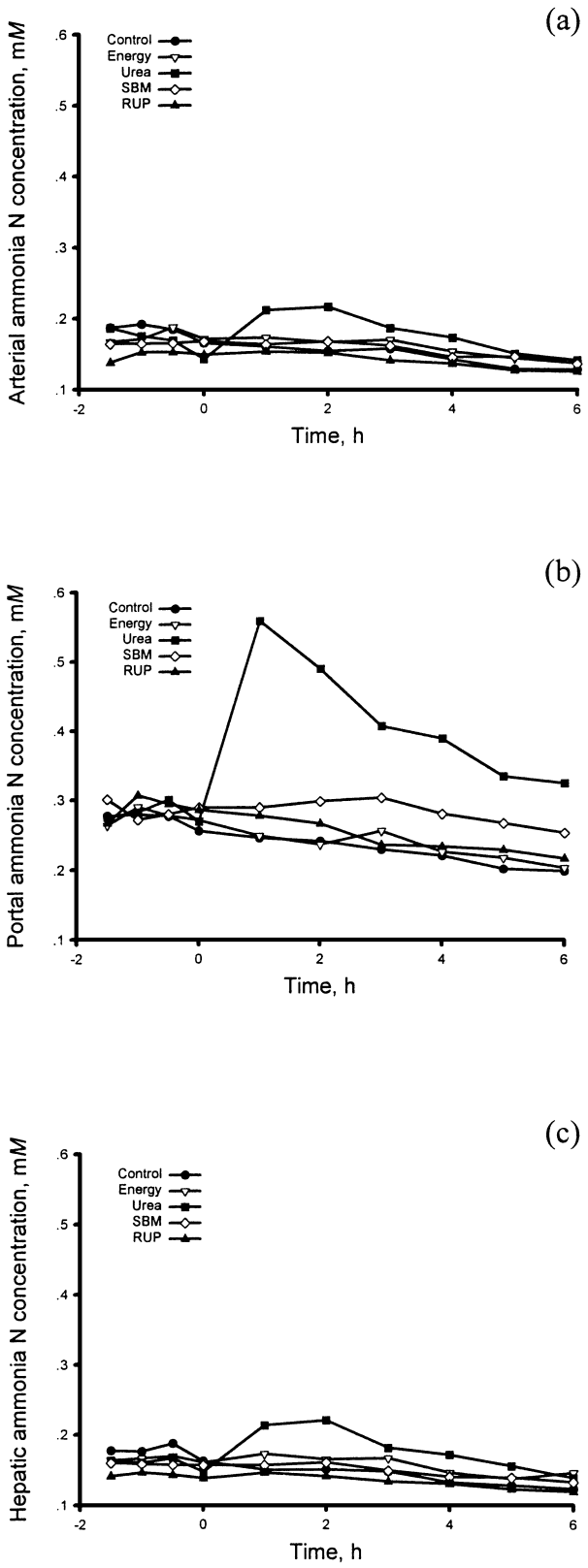


Figure 1. Ammonia N concentrations in arterial (Panel a), portal venous (Panel b), and hepatic venous (Panel c) blood of sheep fed the control diet or the control diet supplemented with energy, energy plus urea (urea), energy plus soybean meal (SBM), or energy plus ruminally undegradable protein (RUP). Statistical analyses are shown in Table 4.

(Figure 2). Net PDV release of ammonia N in sheep supplemented with SBM increased from an average of 16.7 mmol/h prefeeding to 27 mmol/h at 3 h after feeding, then declined. Net PDV release of ammonia N changed little for control or energy- or RUP-supplemented sheep during the sampling period. Presumably, these patterns of PDV release of ammonia N reflect solubility of the nitrogenous sources and rate of degradation and release of ammonia in the rumen. Net uptake of ammonia N by the liver mirrored PDV release (Figure 2) such that a small (and nonsignificant) net splanchnic uptake of ammonia N was observed for all treatments. Net splanchnic uptake of ammonia N did not differ ($P = .93$) among treatments, nor was it significantly influenced by other effects included in the statistical model, suggesting that the liver had sufficient capacity to detoxify the ammonia N at the levels presented.

*Urea N.* Both treatment and hour × treatment interaction were significant sources of variation for urea concentration in arterial, portal venous, and hepatic venous blood (Table 4). As shown in Figure 3, concentrations in the three vessels within treatment followed similar patterns across the sampling period. Concentrations were lowest and stable through time for control and energy treatments. Arterial concentrations averaged 1.81 and 1.89 m*M*, respectively. Likewise, urea concentrations in SBM and RUP treatments were relatively stable through time, but concentrations were greater ($P < .05$) than for control or energy treatments and averaged 3.86 and 3.67 m*M*, respectively. In contrast, urea N concentrations for the urea treatment were lowest prior to feeding (3.23 m*M*) then increased throughout the remainder of the sampling period (5.63 m*M* at 6 h).

Net release of urea N from the liver differed ($P = .04$) among treatments (Table 4). The hour × treatment interaction was not significant ($P = .28$), but the patterns observed are considered to be relevant to the interpretation. Hepatic release of urea N in the control group (26.52 mmol/h) was lowest ($P < .05$) and stable through the sampling period. Hepatic urea N release in sheep fed the energy supplement (40.31 mmol/h) was greater ($P < .05$) than in the control. This observation is consistent with the greater hepatic uptake of $\alpha$-amino N and ammonia N with energy supplementation than in controls. Net hepatic release of urea N was greater ($P < .05$) for urea (65.86 mmol/h) and SBM (56.89 mmol/h) than for other treatments, but the patterns of release for urea and SBM were dissimilar. Values for urea-supplemented sheep averaged 46.83 mmol/h prefeeding then increased to a plateau of approximately 82.22 mmol/h, whereas values for SBM-supplemented sheep averaged 53.18 mmol/h from –1.5 to 2.0 h, increased to 73.98 mmol/h at 3 h postfeeding, then declined. These patterns of urea N release were consistent with patterns of ammonia N release from the PDV and uptake by the liver. Net hepatic release of urea N in RUP-supplemented sheep generally declined from 57.86

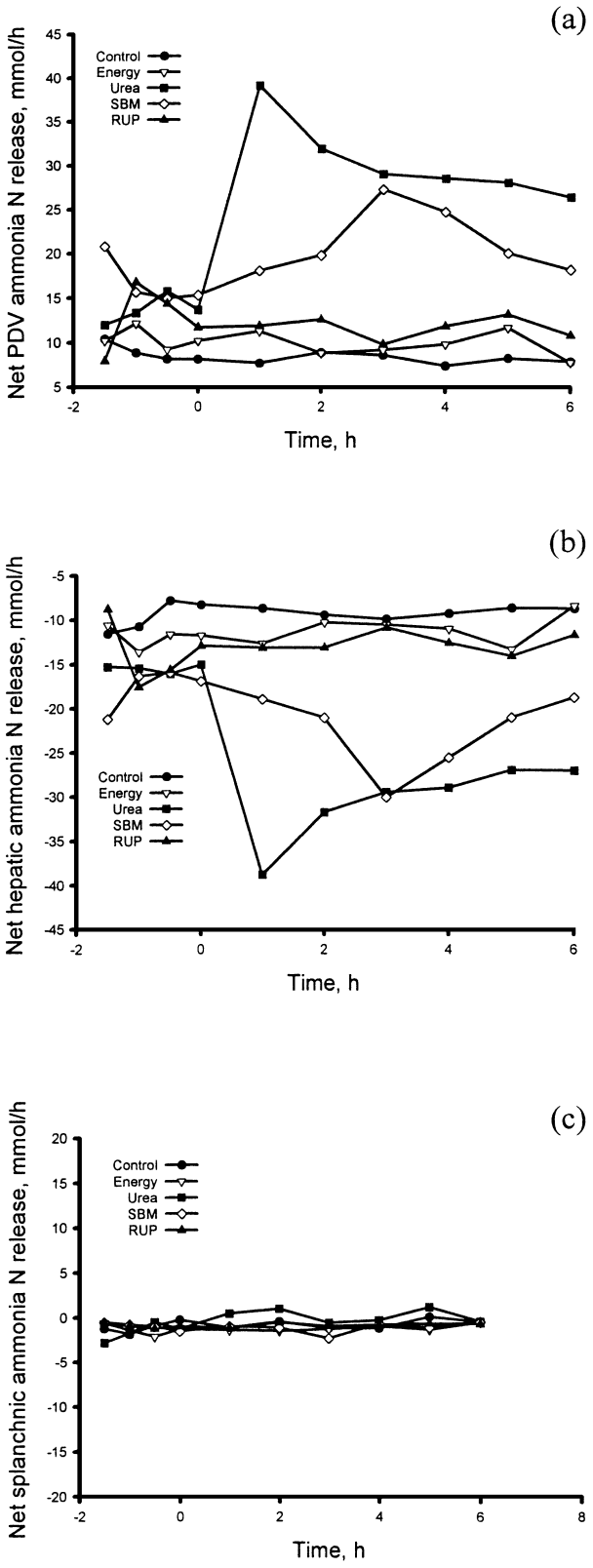


Figure 2. Net release of ammonia N from portal-drained viscera, (PDV, Panel a), hepatic (Panel b), and total splanchnic (Panel c) tissues for sheep fed the control diet or the control diet supplemented with energy, energy plus urea (urea), energy plus soybean meal (SBM), or energy plus ruminally undegradable protein (RUP). Statistical analyses are shown in Table 4.

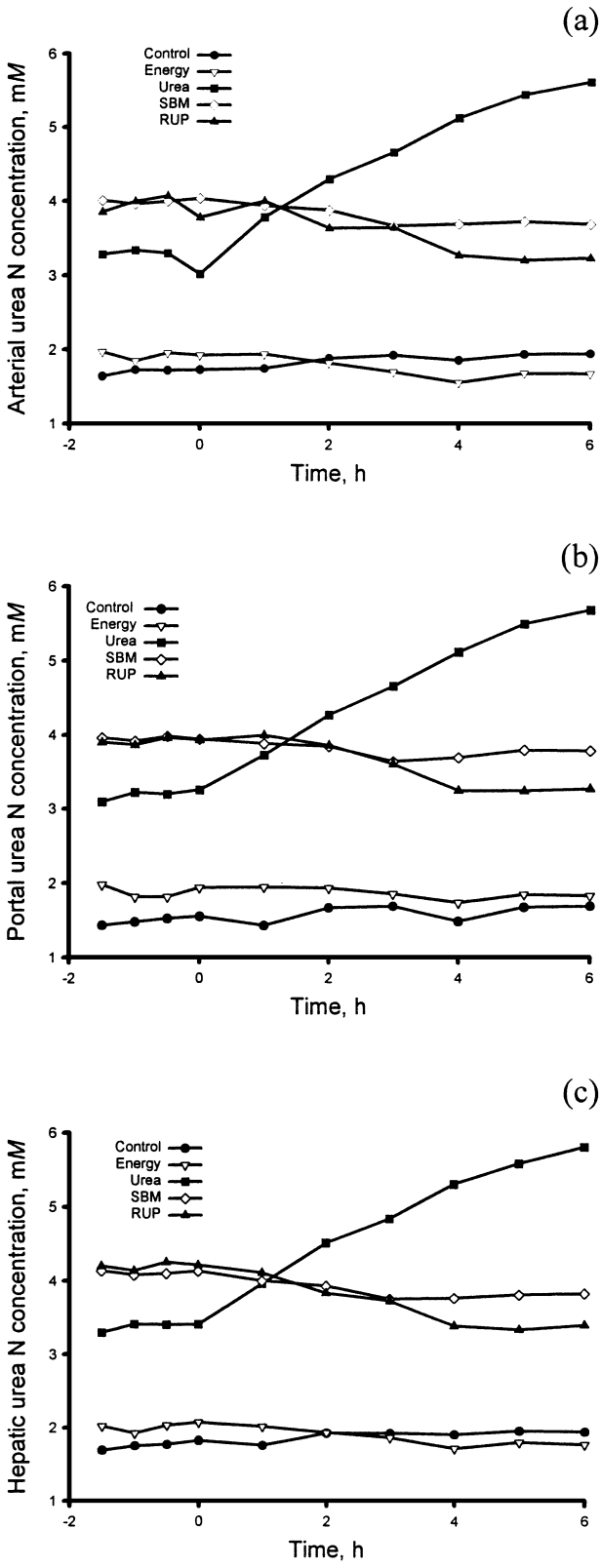


Figure 3. Urea N concentrations in arterial (Panel a), portal venous (Panel b), and hepatic venous (Panel c) blood of sheep fed the control diet or the control diet supplemented with energy, energy plus urea (urea), energy plus soybean meal (SBM), or energy plus ruminally undegradable protein (RUP). Statistical analyses are shown in Table 4.

mmol/h prefeeding to 27.45 mmol/h at 2 h postfeeding, and then they increased to approximately the prefeeding rate of release. The reason for this pattern is not readily evident. Urea N release from the liver agreed favorably with the sum of $\alpha$-amino N and ammonia N uptake by the liver for all treatments.

Although large differences were observed in hepatic urea N release, net uptake by the PDV was not significantly influenced by treatment ($P = .33$) or the hour × treatment interaction ($P = .59$). Mean values were numerically greatest for energy and SBM-supplemented sheep, intermediate for control and urea-supplemented sheep, and least for RUP-supplemented sheep. As a result, treatment ($P = .04$) and the hour × treatment interaction ($P < .03$) were significant sources of variation in net splanchnic release of urea N. Values were low and relatively stable for control and energy-supplemented sheep and averaged 7.08 and 13.98 mmol/h, respectively. Mean release was greater ($P < .05$) for sheep supplemented with RUP (35.91 mmol/h) than for those supplemented with SBM (26.79 mmol/h), but the patterns over the sampling period were similar, in that in both treatments values were high prefeeding, declined until 2 h postfeeding, then increased to approximately prefeeding values. Mean splanchnic tissue release of urea N in urea-supplemented sheep did not differ ($P > .05$) from that in RUP-supplemented sheep, but the pattern of release was very dissimilar. In urea-supplemented sheep, net splanchnic release of urea N averaged 23.5 mmol/h prefeeding, then increased to a plateau of approximately 55.42 mmol/h. The pattern was similar to that observed for ammonia N release from the PDV (Figure 1). The pattern of net release of urea from splanchnic tissues of RUP-supplemented sheep was similar to that described regarding hepatic release.

Net oxygen uptake by hepatic and splanchnic tissues was greater ($P < .05$) in supplemented sheep than in controls (Table 4) and greater ($P < .05$) in sheep fed N plus energy than in those supplemented solely with energy. These results were likely primarily attributable to differences in digestible energy intake among the treatments (Burrin et al., 1989, 1991).

In cattle, increased amino acid flow to the small intestine, either by infusing casein into the abomasum (Guerino et al., 1991; Krehbiel and Ferrell, 1999) or by increasing feed intake (Huntington et al., 1988; Glenn et al., 1989), resulted in greater net PDV release of $\alpha$-amino N. Therefore, the differences in net release of $\alpha$-amino N from the PDV observed in this study likely reflect differences in amino acid flow to the small intestine. In addition, because adding urea to the energy supplement caused a large increase in $\alpha$-amino N release from the PDV, it is evident that dietary N and N recycled to the rumen was inadequate for maximal microbial growth in sheep supplemented with energy. Also, it is noteworthy that $\alpha$-amino N release from the PDV was not greater (numerically less) in sheep supplemented with RUP compared to urea- and SBM-supplemented sheep. This observation, combined with observations that ammonia N release was lower ($P < .05$) and urea N recycling to the PDV was numerically smaller in the RUP-supplemented sheep, suggests that microbial metabolism and growth in the rumen, hence microbial protein flowing to the small intestine, were likely less in the RUP-supplemented sheep than in those supplemented with urea or SBM.

The importance of recycled N to the animal, particularly when consuming low-protein diets, is reflected by observations (Figure 4) indicating that urea N uptake by the PDV was similar to ingested N in control and energy-supplemented sheep. Urea N recycled to the PDV was much lower, relative to intake, in sheep receiving supplemental urea, SBM, or RUP. Huntington (1986) reported that urea N transfer from the blood to the PDV varies from 10 to 42% of N intake. It is also important to note that urea N may be transferred to the rumen via saliva in substantial amounts and that transfers from the blood involve transfer across intestinal tissues as well as to the rumen (Nolan, 1975). Kennedy and Milligan (1980) noted that rate of endogenous urea transfer to the rumen was associated with ruminal ammonia concentrations, plasma urea concentrations, and the amount of organic matter digested in the rumen. Those associations were less than obvious in these data, however. The simple correlation of net uptake of urea N by the PDV and plasma urea N concentration was .06 ($P = .36$, n = 220), when all data were included. Similarly, the apparently high ruminal ammonia N concentrations in sheep fed the urea (6.6 m*M*) supplement seemed not to have a large negative effect on urea N transfer to the PDV, nor did the relatively low ruminal ammonia N concentrations and high digested organic matter in sheep fed the RUP supplement (4.0 m*M*) have a positive influence on net PDV uptake of urea N.

The importance of recycled N to the N economy is further indicated (Figure 4) by the observation that the sum of net PDV release of $\alpha$-amino N and ammonia N plus fecal N was greater than intake N for all treatments. In fact, net PDV release of $\alpha$-amino N exceeded apparently digested N for control and energy treatments (Tables 3 and 4) and was not substantially lower than apparently digested N for other treatments. The sum of net PDV release of $\alpha$-amino N and ammonia N plus fecal N was slightly less than the sum of intake N plus PDV net uptake of urea N. It seems likely that the difference can be attributed to net release of N from the PDV in other forms such as nucleic acids.

## Implications

Supplementation of a low-quality, bromegrass hay diet with only a source of energy seemed to stimulate mobilization of body protein in sheep. These effects are expected, in the long term, to be detrimental to the animal. With our combination of dietary ingredients, the energy plus ruminal bypass protein supplementation seemed to provide greater digestible energy and

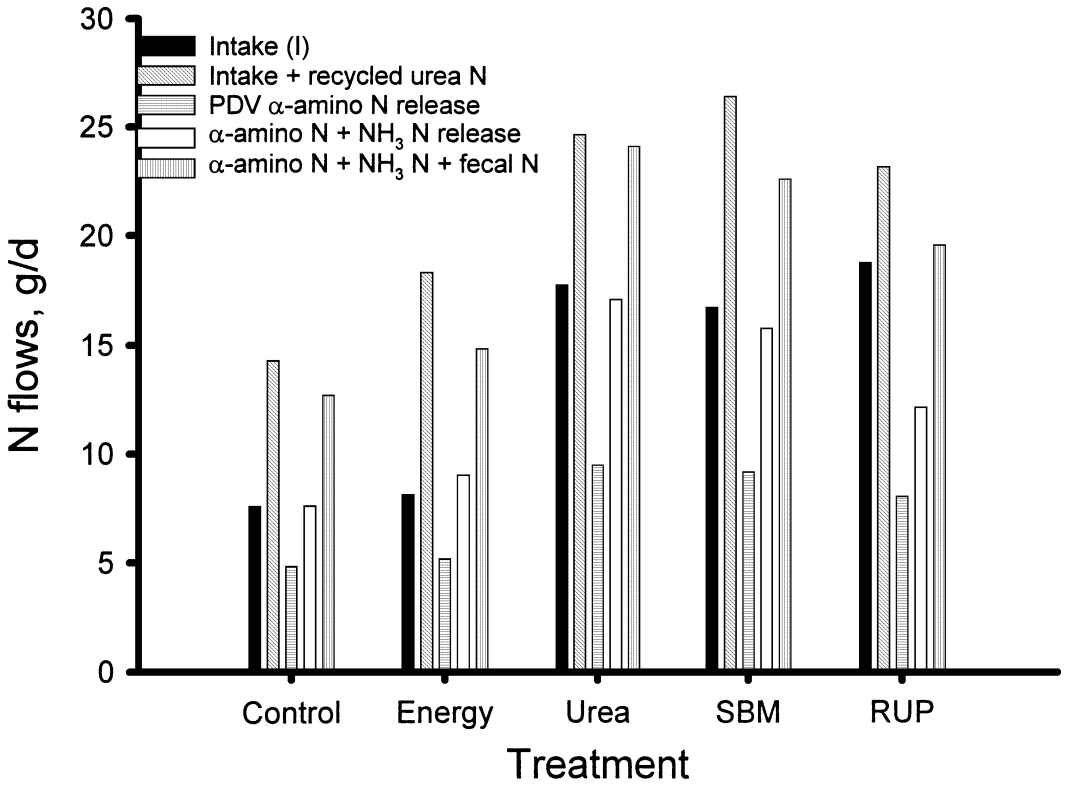


Figure 4. Summary of nitrogen flows in sheep fed the control diet or the control diet supplemented with energy, energy plus urea (urea), energy plus soybean meal (SBM), or energy plus ruminally undegradable protein (RUP). Statistical analyses are shown in Tables 3 and 4.

available amino acids to the animal but seemed to result in less than maximal microbial growth. Supplementation with an energy source and urea or soybean meal, although through different patterns of supply and mechanisms, may result in similar amounts of nutrients being available to the animal, compared with supplementing energy and ruminal escape protein.

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